



**University of
Zurich**^{UZH}

**Zurich Open Repository and
Archive**

University of Zurich
University Library
Strickhofstrasse 39
CH-8057 Zurich
www.zora.uzh.ch

Year: 2011

In vitro gap-directed translesion DNA synthesis of an abasic site involving human DNA polymerases epsilon, lambda, and beta

Villani, G ; Hübscher, U ; Gironis, N ; Parkkinen, S ; Pospiech, H ; Shevelev, I ; di Cicco, G ; Markkanen, E ; Syväoja, J E ; Tanguy Le Gac, N

Abstract: DNA polymerase (pol) ϵ is thought to be the leading strand replicase in eukaryotes, whereas pols λ and β are believed to be mainly involved in re-synthesis steps of DNA repair. DNA elongation by the human pol ϵ is halted by an abasic site (apurinic/aprimidinic (AP) site). In this study, we present in vitro evidence that human pols λ , ϵ , and β can perform translesion synthesis (TLS) of an AP site in the presence of pol ϵ , likely by initiating the 3'OHs created at the lesion by the arrested pol ϵ . However, in the case of pols λ and β , this TLS requires the presence of a DNA gap downstream from the product synthesized by the pol ϵ , and the optimal gap for efficient TLS is different for the two polymerases. The presence of gaps did not affect the TLS capacity of human pol ϵ . Characterization of the reaction products showed that pol λ inserted dAMP opposite the AP site, whereas gap filling synthesis by pol β resulted in single or double deletions opposite the lesion. The synthesis up to the AP site by pol λ and the subsequent TLS by pols λ and β are not influenced by human processivity factor proliferating cell nuclear antigen and human single-stranded DNA-binding protein replication protein A. The bypass capacity of pol ϵ at the AP site is greatly reduced when a truncated form of the enzyme, which has lost the BRCA1 C-terminal and proline-rich domains, is used. Collectively, our in vitro results support the existence of a mechanism of gap-directed TLS at an AP site involving a switch between the replicative pol ϵ and the repair pols λ and β .

DOI: <https://doi.org/10.1074/jbc.M111.246611>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-56814>

Journal Article

Accepted Version

Originally published at:

Villani, G; Hübscher, U; Gironis, N; Parkkinen, S; Pospiech, H; Shevelev, I; di Cicco, G; Markkanen, E; Syväoja, J E; Tanguy Le Gac, N (2011). In vitro gap-directed translesion DNA synthesis of an abasic site involving human DNA polymerases epsilon, lambda, and beta. *Journal of Biological Chemistry*, 286(37):32094-32104.

DOI: <https://doi.org/10.1074/jbc.M111.246611>

Regular Paper:

**In vitro gap directed translesion DNA
synthesis of an abasic site involving human
DNA polymerases γ , λ and β .**

Giuseppe Villani, Ulrich Hubscher, Nadege
Gironis, Sinikka Parkkinen, Helmut Pospiech,
Igor Shevelev, Giulia di Cicco, Enni
Markkanen, Juhani E. Syvaoja and Nicolas
Tanguy Le Gac
J. Biol. Chem. published online July 13, 2011

DNA AND
CHROMOSOMES

Access the most updated version of this article at doi: [10.1074/jbc.M111.246611](https://doi.org/10.1074/jbc.M111.246611)

Find articles, minireviews, Reflections and Classics on similar topics on the [JBC Affinity Sites](#).

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts

Supplemental material:

<http://www.jbc.org/content/suppl/2011/07/13/M111.246611.DC1.html>

This article cites 0 references, 0 of which can be accessed free at

<http://www.jbc.org/content/early/2011/07/13/jbc.M111.246611.full.html#ref-list-1>

IN VITRO GAP DIRECTED TRANSLESION DNA SYNTHESIS OF AN ABASIC SITE INVOLVING HUMAN DNA POLYMERASES ϵ , λ AND β .

Giuseppe Villani¹, Ulrich Hubscher³, Nadege Gironis¹, Sinikka Parkkinen⁴, Helmut Pospiech², Igor Shevelev⁵, Giulia di Cicco¹, Enni Markkanen³, Juhani E. Syväoja⁴ and Nicolas Tanguy Le Gac¹

¹ Institut de Pharmacologie et de Biologie Structurale, CNRS-Université Paul Sabatier Toulouse III, UMR 5089, 205 Route de Narbonne, 31077 Toulouse cedex, France

² Leibniz Institute for Age Research - Fritz Lipman Institute, Beutenbergstraße 11, D-07745 Jena, Germany, and Department of Biochemistry, PO Box 3000, FI-90014 University of Oulu, Finland

³ Institute of Veterinary Biochemistry and Molecular Biology, University of Zürich-Irchel, CH-8057, Zürich, Switzerland.

⁴ Department of Biology, University of Eastern Finland, PO Box 111, FI-80101 Joensuu, Finland

⁵ Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Videnska 1083, 142 20 Prague, Czech Republic

Running title: Gap directed translesion DNA synthesis

Address correspondence to: Nicolas Tanguy Le Gac, IPBS-CNRS, 205 route de Narbonne, F-31077, Toulouse. Tel: 0561175986. Fax: 0561175994. E-mail: ntl@ipbs.fr

DNA polymerase (pol) ϵ is thought to be the leading strand replicase in eukaryotes, while pols λ and β are believed to be mainly involved in re-synthesis steps of DNA repair. DNA elongation by the human pol ϵ is halted by an abasic site (AP site). In this manuscript we present *in vitro* evidence that human pols λ , β and η can perform translesion synthesis (TLS) of an AP site in the presence of pol ϵ , likely by initiating from 3'OHs created at the lesion by the arrested pol ϵ . However, in the case of pols λ and β , this TLS requires the presence of a DNA gap downstream the product synthesized by the pol ϵ and the optimal gap for efficient TLS is different for the two pols. The presence of gaps did not affect the TLS capacity of human pol η . Characterization of the reaction products showed that pol β inserted dAMP opposite the AP site while gap filling synthesis by pol λ resulted in single or double deletions opposite the lesion. The synthesis up to the AP site by pol ϵ and subsequent TLS by pols λ and β are not influenced by human processivity factor PCNA and human single-stranded DNA binding protein RPA. The bypass capacity of pol λ at the AP site is greatly reduced when a truncated form of the enzyme, that has lost the BRCT and proline-rich domains, is used.

Collectively, our *in vitro* results support the existence of a mechanism of gap-directed TLS at an AP site involving a switch between the replicative pol ϵ and the repair pols λ and β .

Chromosomal DNA replication in eukaryotic cells requires three DNA polymerases (pols): pol α , pol δ and pol ϵ . Pol α is the only polymerase that has an associated activity for synthesis of RNA primers and is able to extend from such primers by synthesizing short stretches of DNA (1, 2). Subsequently, processive DNA synthesis is resumed by pol δ and/or pol ϵ . Recent work in yeast supports a model wherein, during normal DNA replication, pol ϵ is primarily responsible for copying the leading-strand and pol δ primarily responsible for copying the lagging-strand (3).

Abasic sites (AP sites) arise frequently by spontaneous hydrolysis of purines in DNA, represent a common intermediate of numerous DNA repair systems and are among the most common endogenous DNA lesions generated during normal cell growth (4, 5).

An AP site poses a serious problem to the advancement of a pol since the modified base has lost its coding capacity. Accordingly, its replication requires the intervention of one or more Y family pols in a process called translesion

synthesis or TLS (for reviews see 6, 7). Recent publications have shown that, among these pols, human pol η was able to insert nucleotides opposite to the AP site and to extend primers further past the lesion *in vitro* (8). Moreover, pol η showed higher abasic lesion bypass capacity *in vivo* than pols ι , κ and Rev1 (9).

Furthermore, it has also been reported that an AP site could be bypassed *in vitro* by pols of other families such as pol α (10), pol δ in the presence of the processivity factor PCNA (11), pols λ and β (12).

Concerning pol ϵ , a limited capacity of TLS of an AP site has been reported for the yeast enzyme (13) but not for its human counterpart which appeared to be blocked mainly at the base preceding the lesion with minor incorporation opposite to it (14).

A widely accepted model of DNA lesion bypass is the polymerase-switching model that is believed to act at the replication fork to enable replication to continue by bypassing DNA lesions that halt the progression of the replicative pols. In this model, protein-protein interactions mediate a pol handoff at the primer-template terminus from the replicative pol to one or more specialized pols. In eukaryotes, this switching appears to be mediated by a monoubiquitinated form of the processivity clamp factor PCNA. A further switch restores the replicative pol to the primer terminus, and accurate synthesis resumes (reviewed in (7)).

On the other hand, a second model, named gap-filling model, can be envisaged to account for TLS-assisted bypass of DNA lesions outside the context of the replication fork and its purpose would be to seal gaps containing lesions resulting from re-priming events or processing of closely spaced lesions on opposite DNA strands (7). In contrast to the polymerase-switching model, the molecular mechanism(s) underlying the gap-filling model are still largely unknown.

In this work we report *in vitro* DNA gap-dependent TLS at an AP site by human DNA repair pols, pol λ and pol β , in the presence of the replicative human pol ϵ . Human pol η can also perform TLS that, however, does not appear to depend on DNA gaps. We also present evidence that TLS by pols λ and β is not influenced by the human processivity factor PCNA and the human single-stranded DNA-binding protein RPA. We

also show that the capacity of pol λ to bypass an AP site is greatly reduced when a truncated form of the enzyme, that has lost the BRCT and proline-rich domains, is used.

Taken together, our *in vitro* results may suggest the existence of a novel pathway of DNA repair, gap-directed TLS involving human pols ϵ , λ and β .

Experimental procedures

Proteins - Recombinant human pol λ , RPA and PCNA were expressed and purified as described (15-17). Recombinant pol λ mutant²⁴⁴⁻⁵⁷⁵ was expressed as described (18) and purified as described (19). Recombinant human pol β was from Trevigen Inc. (Gaithersburg, MD). Recombinant human pol η was from Enzymax (Lexington, KY). Human pol ϵ was purified from HeLa cells through six purification steps as described (14, 20). The glycerol gradient fraction used in this study had a specific activity of 24,000 units/mg. Its purity was estimated to be > 50 % and the fraction was devoid of other replicative pols (14).

DNA substrates and chemicals - The 100-mer oligonucleotide templates, either undamaged or containing a synthetic AP site (tetrahydrofuran moiety), the oligonucleotide primers were from Eurogentec. The oligonucleotides complementary to the 5' end of the templates were from Sigma and all molecules carried a 5' phosphate. All oligonucleotides were PAGE purified. The DNA substrates used in this study are indicated in Table I. Primers were 5'-labelled with T4 polynucleotide kinase (New England Biolabs) in presence of [γ -³²P]-ATP according to the manufacturer's protocol. Each primer was mixed with the templates at a 1:1 (M/M) ratio in presence of 20mM Tris HCl pH 8 and 50mM KCl, heated at 90°C for 5 minutes and then slowly cooled. When necessary, the oligonucleotides complementary to the 5' end of templates were added to the reaction at an oligonucleotide/template ratio of 2:1 (M/M) to insure complete hybridization to all templates. [γ -³²P]-ATP was from Perkin Elmer, dNTPs were from Fermentas Life Science and ddGTP was from GE Healthcare. 20X Glycerol Tolerant Gel (GTG) buffer was from USB.

Primer extension assays - Reaction solutions of 10 μ l were incubated at 37°C and contained 0.15 picomole of DNA templates, 50mM Hepes pH 7.5,

5mM MgCl₂, 1mM DTT, 200µg/ml of BSA and 100µM each of dATP, dCTP, dGTP and dTTP. The incubation times and the amount of proteins used are indicated in the legends of the figures. The reactions were stopped by adding 5µl of stop solution containing 0.1% xylene cyanol and 0.1% bromophenol blue in 90% formamide. Before loading onto the gel, samples were denatured by heating at 100°C for 3 min. The reaction products were resolved on denaturing polyacrylamide gel electrophoresis (7M urea, 10% acrylamide) run in GTG buffer (90mM Tris pH 9, 30mM taurine and 5 mM EDTA) and visualized and quantified using Molecular Dynamics PhosphorImager and ImageQuant software.

The percentage of translesion synthesis (TLS) was calculated as the ratio of the intensity of bands present at the position opposite the lesion or beyond to the intensity of these bands plus the one present one nucleotide before the lesion.

DNA sequencing of reaction products- Reaction solutions of 10µl were incubated at 37°C and contained 50mM Hepes pH 7.5, 5mM MgCl₂, 1mM DTT, 200µg/ml of BSA and 100µM each of dATP, dCTP, dGTP and dTTP and 0.15 picomole of DNA templates containing a single AP site within either a 2-nucleotide-gap or a 4-nucleotide gap. 2-nucleotide and 4-nucleotide gap templates were replicated by pol ε for 15 minutes followed by addition of pol β or pol λ for 5 minutes, respectively. Gap-filled products were converted to 100-mer by addition of 10U of T4 DNA ligase and incubation at 37°C for 10 minutes. Ligated products were amplified by 30 cycles of PCR in the presence of 1.25U of Pfu DNA polymerase (Fermentas) and 25pmoles of both 5'-ACTACATTTACTTTCAATTACATAATTTCA AATCCTAATAATCT-3' and 5'-TAAGGTAGTAGTATTATAAATTATG-3' primers. PCR-amplified products from three independent reactions were pooled and purified. 29 and 28 individual clones were sequenced after TOPO-cloning of the purified products for pols β and λ respectively (Millegen, Toulouse, France).

Gel mobility-shift assay - Reaction of 10µl contained 0.3 picomoles of DNA substrates and 1.5 picomoles of pol β in 50mM Hepes pH 7.5, 5mM MgCl₂, 1mM DTT and 200µg/ml of BSA. After incubation time of 15 minutes at 37°C, 1µl of loading buffer (50% glycerol, 0.20% bromophenol blue, 0.20 % xylene cyanol and

0.2M EDTA) was added and the mixture loaded on a 8% native gel in 0.5 X TBE running buffer (89mM Tris/borate, pH 8.3 and 2mM EDT) and electrophoresed at 4°C at 10V/cm for 3 hours.

RESULTS

DNA polymerase λ requires a DNA gap with a specific length to perform translesion synthesis in the presence of DNA polymerase ε. DNA elongation by human pol ε is severely blocked by an abasic site (14) while human pol λ can bypass such DNA lesion (12). We therefore investigated whether pol λ could resume DNA synthesis when pol ε was stalled at an AP site. For initial experiments the 100-mer template shown in Table 1a was used. The template contains a unique synthetic AP site at a defined position and it was annealed to a primer of 44-mer, since a minimal primer length of ≈40 bp is required to maximize the binding and processivity of pol ε (21). As previously reported (14), when incubated with the 100-44 mer template-primer (that is defined in this work as single stranded template-primer) pol ε was unable to replicate past the abasic site and stopped primarily at the base preceding the lesion, with some incorporation opposite the lesion (lane 2 of Fig. 1A, quantified in Fig. 1B).

Addition of pol λ (0.25 picomoles) at this stage and further incubation for 5 minutes did not resume DNA synthesis (Fig. 1A, lane 4).

Pol λ is a family X pol that is involved in DNA repair and has higher incorporation efficiency on gapped than single stranded DNA (see (22) and ref therein). We therefore constructed template-primers containing gaps of different lengths around the abasic site and tested the capacity of pol λ to perform TLS in presence of pol ε. To this purpose we hybridized the 100:44 mer single stranded template-primer with oligonucleotides of different length placed downstream the abasic site. With these template-primers arrest of elongation by pol ε at the AP site results in gaps of a size from 1 to 13 nucleotides starting from the base that follow the abasic site (Fig. 1a). Since pol λ possesses a dRP lyase activity and it has been shown that a 5'phosphate present in a gap strengthens the binding of the enzyme (23), all oligonucleotides placed downstream the AP site were synthesized with a 5' phosphate.

As it can be seen in Fig. 1A, DNA gaps of 2, 4 and 6 nucleotides starting from the lesion radically changed the behaviour of pol λ that now acquired the capacity to perform TLS of the AP site in the presence of pol ϵ (see lanes 7, 10 and 13). The gaps enabled pol λ to catalyse both incorporation opposite the lesion and beyond and to fill the gaps with no or little strand displacement synthesis consistent with the known limited strand displacement capacity of the enzyme (19) and see also Fig. 2). The TLS capacity of pol λ appears to increase from gaps of 2 nucleotides to gaps of 4 to 6, but is dramatically reduced with a gap of 13 nucleotides (see quantifications in Fig.1B), where the enzyme almost behaved as with the single stranded template-primer in lane 4. Thus it appears that the TLS capacity of pol λ is strongly modulated by the size of the DNA gap.

As seen in Fig.1, when 0.25 picomoles of pol λ were incubated for 5 minutes with the substrates in the absence of pol ϵ , the products synthesized were all too short to reach the lesion (lanes 3, 6, 9, 12 and 15). This finding suggested that pol λ catalyzed TLS by using the 3'OHs created by pol ϵ , arrested either at the base preceding the lesion or opposite to it, rather than using shorter primers generated during its own synthesis.

To further clarify this point, an experiment was devised in which the template-primers shown in Table I b were used. If one compares template 1a to 1b it can be seen that in the latter the sequence between the 3'-OH of the primer and the AP site has been changed, so that the only Cs present in the template are now within this sequence. This allows specific inhibition of any elongation from 3'OHs in this region when the chain elongation inhibitor ddGTP is used. Oligonucleotides were hybridized to this template-primer to create gaps of 4 and 13 nucleotides respectively. The rationale of this approach is that simultaneous addition of both pol λ and ddGTP in the presence of the stalled pol ϵ will abolish any priming contribution not starting from a pre-existing 3'-OH. The result of this experiment is shown in Suppl. Fig. 1. Note that to maximize the inhibitory effect of ddGTP, a concentration of 1 picomole of pol λ instead of the usual 0.25 picomoles was used. As expected, incubation of 1 picomole of pol λ for 5 and 10 minutes in the absence of pol ϵ resulted in increased synthesis compared to that seen previously with 0.25 picomoles (compare lanes 3

and 4 of Suppl. Fig. 1 to lane 3 of Fig. 1). Samples were incubated for 5 and 10 minutes, and at 10 minutes one can see some TLS due to the intrinsic AP bypass activity of pol λ . Addition of ddGTP restrained incorporation to the first G following the 3'OH of the primer (lanes 5 and 6). On single stranded template-primer, addition of 1 picomole of pol λ in the presence of pol ϵ induced some TLS, particularly at 10 minutes, and this is seen also for the template-primer containing the 13 nucleotides gap (lanes 7, 8 and 15, 16). However addition of ddGTP before addition of pol λ completely abolished this TLS, indicating that it was due to elongation by pol λ of pre-existing primers and not of those created by pol ϵ (lanes 9, 10 and 17, 18). Interestingly, the situation appeared different with the template-primer containing the 4 nucleotides gap, where a substantial part of the TLS by high amount of pol λ took place also in the presence of ddGTP (compare lanes 11, 12 to 13, 14), indicating that in this case pol λ could effectively use 3'OHs created at the lesion by pol ϵ .

To directly confirm that pol λ could extend past the lesion in presence of a gap starting from 3'OH generated by pol ϵ , as suggested by the experiments shown in Fig. 1 and Suppl. Fig.1, we created two template-primers in which the primer is either a 45 mer bearing a 3'OH at the base preceding the AP site or a 45 mer bearing the 3'-OH at an A placed opposite the lesion (Table I, template-primers 1c). The choice of the latter template-primer was motivated by our previous results which showed that either A or C is incorporated by pol ϵ opposite the AP site (14). To these template-primers, appropriate oligonucleotides were hybridized to generate gaps of 2, 4, 6 and 13 nucleotides, and they were used in reactions with 0.025 picomoles of pol ϵ and 0.25 picomoles of pol λ .

Fig. 2A shows the results of reactions using the primer ending at the nucleotide preceding the AP site. As expected, pol ϵ can incorporate in front of the lesion but is unable to elongate past it with all the substrates tested (lanes 2-6). On the contrary, pol λ alone can replicate past the AP site when gaps are present and, importantly, with a gap preference similar to that observed when the 3'OHs were generated by the arrest of pol ϵ at the lesion (compare lanes 9-12 of Fig. 2A to lanes, 7, 10, 13 and 16 of Fig. 1A).

Fig. 2 B shows the results of reaction with the primer bearing an A opposite the AP site. As it can be seen, pol ϵ is unable to elongate from this nucleotide, likely because its 3' to 5' exonuclease continuously excised the A, as indicated by the increased intensity of the band preceding the lesion (compare lanes 2-6 of Fig.2A to 2-6 of Fig. 2B). Unlike pol ϵ , pol λ can also replicate from the A opposite the AP site, again with a gap preference similar to the one observed in Fig 1. It should be noted that, as shown in Fig.1, the amount of λ used in our study essentially fills the gaps during TLS with no significant strand displacement synthesis.

Taken together these data strongly suggest that the presence of gaps of defined size is a major determinant allowing pol λ to substitute for pol ϵ in order to bypass the AP site (see also Discussion). Related to our finding, it is interesting to note that a recent work has shown that the polymerization activity of human pol λ increases with DNA gaps from 1 to 4 remains constant for gaps from 4 to 7 nucleotides and then drops for gaps from 7 to 10 nucleotides (22).

The full length DNA polymerase λ is required for the AP site translesion synthesis in the presence of DNA polymerase ϵ . Pol λ has two non-enzymatic domains at its N-terminus: a BRCA1 C-terminal (BRCT) domain and a proline rich domain (for review see (23)). Little is known about the functions of these domains, but BRCT domains are known to mediate protein-protein and protein-DNA interactions (24) and both domains have been suggested to up-regulate or down-regulate fidelity of pol λ during gap filling activity, depending on the length of the gaps (22, 25). We therefore compared the AP site TLS capacities of pol λ wt and of the mutant form missing the BRCT and proline rich domains ($\lambda^{244-575}$) in the presence or absence of pol ϵ . The results of this comparison are shown in Figs. 3 and 4. Fig. 3A, quantified in B, shows that the $\lambda^{244-575}$, with both the 4 and 6 nucleotide gaps depicted in Table 1a, had a severely reduced capacity to perform AP site translesion synthesis in presence of pol ϵ compared to the wt (compare lanes 5 to 6 and lanes 7 to 8). Note that the two enzymes displayed the same activity on single-stranded template-primer (lanes 3 and 4 of Fig. 3A). Next, we examined the capacity of the two forms of pol λ to

replicate past the AP site when initiating elongation from a 3'OH preceding the lesion, using the 100/45 template-primer shown in Table 1c. As can be seen in Figs 4A and B, pol $\lambda^{244-575}$ had also a clearly diminished intrinsic capacity to bypass the AP lesion compared to the wt (compare lanes 4 to 5 and lanes 6 to 7). Note that neither wt nor mutant pol λ could replicate past the AP site on single-stranded template-primer or on a template bearing a gap as long as 13 nucleotides (see lanes 2, 3 and 8, 9 of Fig. 4A, quantified in B), in agreement with the results previously shown.

These experiments show, first, that a pol λ mutant lacking its BRCT and proline-rich domains has an impaired capacity to perform TLS of an AP site in the presence of pol ϵ , and, second, that this defect can be attributed to an intrinsic diminished capacity to bypass the lesion, therefore suggesting a role of these domains in facilitating TLS of an AP site by pol λ .

DNA polymerase β and DNA polymerase λ show different DNA gap size preference for translesion synthesis past an AP site in the presence of DNA polymerase ϵ . Next we tested whether DNA gap sizes could influence, in the presence of pol ϵ , TLS of an AP site by pol β , another X family repair polymerase that displays high affinity for very short DNA gaps. A direct comparison between the TLS capacity of β and λ as a function of DNA gap size is presented in Fig. 5.

First, it should be noted that, on a single stranded template-primer and at the same protein concentration (0.25 picomoles) pol β could synthesize up to the AP site while pol λ could not (compare lanes 5 and 6 to lanes 3 and 4 of Fig. 5A). However, the elongation was blocked at the base preceding the lesion. Therefore it appears that pol β cannot bypass the AP site when acting alone on a single stranded template-primer. Furthermore, Fig 5A also shows that neither pol λ nor β can perform TLS by utilizing primers created by pol ϵ in a single stranded context (lanes 7, 8 and 13, 14, quantified in Fig. 5B). In the presence of pol ϵ and in agreement with the data shown in Fig 1, pol λ could easily replicate past the AP site when the gaps were 2 or 4 nucleotides long (lanes 9,10 and 11, 12).

Interestingly, pol β also showed the capacity to bypass an AP site in the presence of pol ϵ , but only

when the gap was 2 nucleotides long; enlarging the gap to 4 nucleotides abolished the TLS capacity of pol β (lanes 15, 16 and 17, 18). Pol β appeared to be less efficient than pol λ in TLS of a 2 nucleotides gap (see quantifications in Fig. 5B and C), which could be due to the superior intrinsic capacity of pol λ to replicate an AP site (12).

The different gap size dependency of TLS between pols λ and β (see also Figs 7 and 8) corresponds to the preferences of the respective pols for undamaged substrates. Pol β fills preferentially gaps of one nucleotide and its incorporation efficiency decreases with the increase in gap size from 1 to 4 nucleotides (22). To directly investigate the affinity of pol β to the templates-primers used, we measured its binding capacity to substrates containing AP sites in gaps of 1, 2, 3, 4, 6 and 8 nucleotides. These substrates were created by first annealing a 66 mer primer to the 100 mer containing the AP site, so that the primer ended at the base preceding the AP site, as depicted in Table Id. Then the appropriate oligonucleotides were annealed downstream the lesion to create the substrates mentioned above. The experiment in Supp. Fig. 2 shows that the amount of Pol β bound to the substrate was highest with a 1 nucleotide gap and diminished with increasing gap size. The reduction in binding efficiency parallels the decrease of TLS by pol β in the presence of pol ϵ , indicating that the TLS capacity of the enzyme is directly correlated with its binding capacity at gaps.

Nucleotide insertion opposite the AP site by polymerases β and λ during gap-directed translesion synthesis.

To determine the identity of the nucleotide inserted opposite the synthetic abasic site by pol β and λ , we have ligated the elongated primers to the downstream oligonucleotide and amplified the full-length products by PCR (see Experimental procedures). Since Pfu DNA polymerase is blocked by the synthetic AP site (data not shown), only the newly synthesized DNA strand is amplified during the PCR reaction. As shown in Supp. Table I, sequencing of individual clones revealed that pol β TLS of the AP-site in a 2-nucleotide DNA gap context resulted in incorporation of dAMP opposite the AP-site in all

the products sequenced, according to the proposed model of bypass of an abasic lesion by pol β (26). On the other hand, 4-nucleotides DNA gap filling reactions by pol λ resulted in single or double deletions opposite the lesion, according to the known misalignment capacity of the enzyme (27). It should be noted that, in our template sequence, the AP-site is followed by a run of 4 thymine residues; therefore, incorporation of dAMP by pol λ opposite the lesion followed by primer template slippage and annealing to a downstream thymine can lead to the observed pattern.

Translesion synthesis of an AP site by DNA polymerase η , acting alone or in the presence of DNA polymerase ϵ , is not influenced by the presence of DNA gaps. Next we investigated the capacity of the human pol η to perform TLS of an AP site either in the presence of pol ϵ or when acting alone (Fig. 6). 0.25 picomoles of pol η , incubated with single-stranded template-primer in a running start reaction, synthesized up to the AP site and, differently from pol ϵ , efficiently incorporated opposite the lesion (compare lane 4 to lane 2 of Fig 6A and data not shown). When the reaction was performed in the presence of pol ϵ , pol η performed TLS with the single-stranded template primer, as indicated by the increase in incorporation in front of the lesion and the appearance of some full length products (see lane 9 of Fig. 6A). However, this TLS was diminished in the presence of gaps of 2, 4 and 6 nucleotides and extension past the lesion was not affected by the increase in their size (see lanes 10 to 12 of Fig. 6A). This result was different from what observed with pol λ , where no TLS was observed in the presence of pol ϵ with single-stranded template primer (see lane 5 of Fig. 6A) while efficient gap filling was performed with DNA gap sizes of 2, 4 and 6 nucleotides (see lanes 6 to 8 of Fig 6A).

We have also studied the TLS capacity of pol η when acting alone on gaps created in a template-primer where the primer is a 45-mer with a 3'OH at the base preceding the lesion. As shown in Fig. 6B, pol η can perform TLS on the single-stranded template (lane 7) but to a lesser extent with gaps of 1, 2, 4 and 6 nucleotides, with no increase in efficiency with the increasing gap size (see lanes 8 to 11). Conversely, TLS by pol λ was stimulated with gap size increasing from 2 to 6 nucleotides (lanes 4 to 6). Note that pol η showed little TLS

also with gap size of 1 and 2 nucleotides that are optimal for TLS by pol β (see Fig. 8).

Taken together these results show that, differently from pol λ , TLS of an AP site by pol η is not stimulated by the presence of DNA gaps.

PCNA and RPA do not influence the gap size preference of DNA polymerases λ and β to perform translesion synthesis of an AP site in the presence of DNA polymerase ϵ . The processivity factor PCNA and the single-stranded DNA-binding protein RPA play a fundamental role in DNA replication, repair and recombination (2, 5). Therefore we studied whether the TLS catalysed by pols λ and β at DNA gaps in presence of pol ϵ could be influenced by human PCNA and RPA. To this aim we performed experiments by adding 1.2 picomoles of PCNA and 0.25 picomole of RPA. With respect to the 0.15 picomole of template-primer used in the study, the RPA concentration corresponds to roughly one molecule of RPA for 30 nucleotides of single-stranded DNA.

Fig 7 shows the results with pol λ . As it can be seen with a wide range of DNA gaps (Fig. 7A) pol λ performed TLS with an efficiency dictated by the size of the DNA gaps. This efficiency increased with gaps from 1 to 4 nucleotides, remained constant with 6 and 8 nucleotides and then declined to an almost undetectable level with the gap of 13 nucleotides, as quantified in Fig. 7B. As previously shown, also in this reaction containing both PCNA and RPA, the TLS products length essentially matched the size of the gaps, although low levels of strand displacement could also be detected.

Fig. 8 shows the results with pol β . As it can be seen, the TLS by pol β is restricted to gaps of 1 and 2 nucleotides, becoming almost undetectable with a gap of 4 nucleotides or longer (Fig. 8A). In agreement with the experiments described previously, the efficiency of TLS by pol β was lower when compared to that observed with pol λ (quantified in Fig. 8B) and no strand displacement was observed.

DISCUSSION

Pols δ and ϵ are the two eukaryotic pols that replicate DNA. Evidence in yeast supports the conclusion that pol δ is primarily responsible for

copying the lagging-strand while pol ϵ primarily copies the leading-strand (3). In addition, these pols also participate in DNA repair processes such as nucleotide excision repair (NER) long patch base excision repair (LP-BER) and mismatch repair (MMR) (2).

Apurinic or apyrimidinic (AP) sites are the most frequent spontaneous lesions in DNA. A number of pols, belonging mainly but not exclusively to the Y family pols, can perform *in vitro* TLS of an AP site (9, 28). Pols λ and β , two pols of the X family believed to be implicated in re-synthesis steps of DNA repair, can also bypass an abasic site (12).

Although much information is available in literature concerning the capacity of pol δ to deal with an AP site (11, 29), such information is scarce for pol ϵ , particularly for the human enzyme. In a recent publication we have shown that *in vitro* elongation by human pol ϵ stopped predominantly at the base preceding the lesion with roughly 10% of residual incorporation opposite to it (14).

As indicated in the Introduction, it is generally accepted that during TLS specialized polymerases replace arrested replicative pols at lesions to allow bypass.

We have investigated the ability of human pols λ , β and η to perform TLS of an AP site in the presence of short DNA gaps created by the arrest of human pol ϵ at the lesion. To this aim we have set up a system in which pol ϵ synthesizes on a DNA oligonucleotide template-primer leaving gaps with lengths spanning from 1 to 13 nucleotides due to stalling at the AP site (Table I). Pol λ cannot bypass an AP site in the presence of Pol ϵ if a single stranded stretch of 35 nucleotides is present downstream the lesion, but can do it with gaps from 1 to 10 nucleotides, with a maximum efficiency around 4 to 6 and with almost no TLS with a gap of 13 nucleotides (Figs 1, 3, 5 and 7). Furthermore, our data indicate that: a) pol λ can bypass an AP site by using the 3'OHs generated by the arrest of pol ϵ at the lesion (Suppl. Fig. 1); b) it can act alone to bypass the AP site only when the lesion is present in a gap context and, importantly, with a gap preference similar to the one observed in the presence of pol ϵ (Fig. 2); c) the BRCT and proline-rich domains of the pol λ are required for efficient TLS of the lesion (Figs. 3 and 4). These results show that pol

λ can replace pol ϵ and bypass an AP site utilizing 3'OHs created by the arrest of pol ϵ at the base preceding the lesion or opposite to it. Most interestingly, this switch can take place only on short DNA gaps, whose size strongly influences the efficacy of the process, and bypass of an AP site requires the presence of the BRCT and proline-rich domain of the pol λ . We attempted to further characterize this scenario by investigating directly the binding capacity of pol λ with the gapped DNA substrates used in this study. In contrast to experiments with pol β (see below), we were unable to detect stable interaction between pol λ and DNA substrates under a variety of conditions. However, our results are in full agreement with the gap size preference of pol λ on undamaged DNA recently published (22).

Pol β can also catalyze TLS of an AP site in the presence of pol ϵ , but only if the gaps have a size of 1-3 nucleotides, with an efficacy of $1 > 2 > 3$ nucleotides and with no bypass of an AP site at gaps longer than 3 nucleotides (Figs. 5, 8 and data not shown). This preference parallels the binding affinity of pol β for the DNA substrates, as it can be seen in Suppl. Fig. 2, and is in agreement with the fact that its incorporation efficiency for undamaged substrates is the highest with 1 nucleotide gap and then decreases with the increase of gap size (22). The efficiency of TLS by pol β appears to be reduced compared to the one displayed by pol λ , likely due to the superior intrinsic capacity of pol λ to replicate an abasic site (12).

Sequencing of the replication products revealed that pol β exclusively inserts dAMP in front of the abasic site in the context of a 2-gap substrate while pol λ bypass of an AP site in a 4-gap substrate induces single or double deletions (Suppl. Table 1). Insertion of dAMP by pol β is in accordance with a previous model of pol β translesion synthesis of an abasic site that revealed its predisposition to inserting a nucleotide complementary to the first downstream templating base, which is a thymine in our template sequence (26). The observed behaviour of pol λ fits with the scrunching gap-filling model derived from crystal structures of the ternary DNA-pol λ -dNTP complex (30).

Pol η was also proficient in TLS of an AP site when acting alone or in the presence of pol ϵ .

However its TLS capacity was not stimulated by the presence of DNA gaps (Fig 6).

PCNA increases the processivity of the replicative pol δ (2) but its capacity to stimulate or not the processivity of pol ϵ remains controversial, possibly depending on the type of DNA substrates and experimental conditions used (31-34). Furthermore, PCNA has been shown to stimulate TLS of an AP site by pol λ when placed on a 73 mer template, 31 nucleotides away from the 17 mer primer (35).

Since PCNA and RPA play a fundamental role in DNA transactions such as DNA replication, repair and recombination, we set up to study whether the TLS catalysed by pols λ and β at DNA gaps in the presence of pol ϵ would be influenced by these proteins. When compared to previous Figures, Figs. 7 and 8 show that PCNA and RPA had no effect. In addition, Figs. 7 and 8 summarize the major findings of this work by showing that human Pols λ and β can perform TLS of an AP site in the presence of human pol ϵ , PCNA and RPA only in DNA gaps no longer than 10 nucleotides. However, the two pols show distinctly different DNA gap size preference and efficiency in performing such TLS.

Accordingly, our results suggest the simple model shown in Fig. 9, in which the different role of pol β and λ in extending primers generated by pol ϵ arrested at an AP site can be visualized. When long single stranded DNA stretch is present downstream the lesion, neither pol has sufficient affinity for the DNA substrate to displace pol ϵ and continue DNA synthesis past the AP site (Fig. 9a). When the gap downstream the lesion is only 1 or 2 nucleotides long, both pols β and λ could use their affinity for such gaps to bind and perform TLS (Fig. 9b). If the gap size is between 4 and 10 nucleotides, only pol λ would have the capacity to bind and extend past the lesion (Fig. 9c). If the gap size is larger than 13 nucleotides, neither λ nor β could bind productively and we are back to the initial situation with single stranded DNA downstream the AP site (Fig. 9d).

What might be the physiological significance of our *in vitro* observations? At least two possibilities exist. The first concerns DNA repair, namely NER and LP-BER pathways. In mammalian cells it has been shown that pol ϵ can fill the gap of about 27 nucleotides that is produced during NER in a

reaction that includes PCNA and RPA (36). The redundant roles of pols δ and ϵ have been confirmed during NER of a defined lesion reconstituted with recombinant or highly purified factors *in vitro* (37). The requirement of pol ϵ and RPA in the re-synthesis step of NER in human cells has been further demonstrated recently, together with another pathway involving pols δ and κ (38). Since AP sites are among the most frequent endogenous lesions, it is possible that the removal of the damaged 27 mer during NER uncovers an AP site in the DNA template sequence of the gap to be filled. This gap filling will be performed first by pol ϵ up to the lesion and subsequently by pol λ or β depending on the distance of the AP site from the 5' end of the gap. A similar situation can arise during the long-patch BER pathway. It has been suggested that either pol ϵ or pol δ catalyzed elongation during long-patch BER synthesis (39), and it has been shown that both pols can participate in the re-synthesis step of long-patch BER (40). Furthermore, it is now known that two clustered DNA lesions enhance the mutagenicity of individual lesions (for review see (41)). This observation suggests that the delayed repair at one lesion due to the initiation of repair on the opposite strand can lead to mutagenic TLS of the unrepaired damage. In mammalian

cells pols ϵ , β or λ could sequentially perform the replication of an AP site during LP-BER of clustered DNA lesions in the way suggested by our model.

The second possibility concerns gap-directed TLS in connection with DNA replication where small single-stranded DNA gaps accumulate along replicated duplexes. Such gaps may arise either by re-priming of the leading strand (42), or the initiation of a new Okazaki fragment (42, 43). Gap-directed lesion bypass has the advantage that the TLS may be separated from the fork progression, and in fact, recent results suggest that a considerable fraction of TLS occurs in the G2/M phase of the cell cycle, when DNA replication has essentially completed (44). As in repair-dependent, gap-directed TLS, the nature of the lesion and the size of the gap may determine which pol could be best suited for the bypass.

In summary, although based on *in vitro* experiments, our study suggests the existence of DNA gap-directed TLS of an abasic site involving human pols ϵ , λ and β and might serve as a working model for further investigations.

REFERENCES

1. Kornberg, A., and Baker, T. A. (2005) *DNA Replication*, 2nd Ed. University Science Books
2. Hübscher, U., Spadari, S., Villani, G., and Maga, G. (2010) *DNA Polymerases: Discovery, Characterization and Functions in Cellular DNA Transactions*, 1st Ed. World Scientific Publishing Company
3. Kunkel, T. A., and Burgers, P. M. (2008) *Trends Cell Biol.* **18**, 521–527
4. Lindahl, T. (1993) *Nature* **362**, 709–715
5. Friedberg, E. C., Walker, G. C., Siede, W., Wood, R. D., Schultz, R. A., and Ellenberger, T. (2005) *DNA Repair And Mutagenesis*, 2nd Ed. ASM Press
6. Prakash, S., Johnson, R. E., and Prakash, L. (2005) *Annu. Rev. Biochem.* **74**, 317–353
7. Waters, L. S., Minesinger, B. K., Wiltrout, M. E., D'Souza, S., Woodruff, R. V., and Walker, G. C. (2009) *Microbiol. Mol. Biol. Rev.* **73**, 134–154
8. Choi, J., Lim, S., Kim, E., Jo, A., and Guengerich, F. P. (2010) *J. Mol. Biol.* **404**, 34–44
9. Sherrer, S. M., Fiala, K. A., Fowler, J. D., Newmister, S. A., Pryor, J. M., and Suo, Z. (2011) *Nucleic Acids Res.* **39**, 609–622
10. Shibutani, S., Takeshita, M., and Grollman, A. P. (1997) *J Biol Chem* **272**, 13916–13922
11. Mozzherin, D. J., Shibutani, S., Tan, C. K., Downey, K. M., and Fisher, P. A. (1997) *Proc Natl Acad Sci USA* **94**, 6126–6131
12. Blanca, G., Villani, G., Shevelev, I., Ramadan, K., Spadari, S., Hübscher, U., and Maga, G. (2004) *Biochemistry* **43**, 11605–11615

13. Sabouri, N., and Johansson, E. (2009) *J Biol Chem* **284**, 31555–31563
14. Locatelli, G. A., Pospiech, H., Tanguy Le Gac, N., van Loon, B., Hübscher, U., Parkkinen, S., Syväoja, J. E., and Villani, G. (2010) *Biochem. J.* **429**, 573–582
15. Shevelev, I., Blanca, G., Villani, G., Ramadan, K., Spadari, S., Hübscher, U., and Maga, G. (2003) *Nucleic Acids Res.* **31**, 6916–6925
16. Henriksen, L. A., Umbricht, C. B., and Wold, M. S. (1994) *J Biol Chem* **269**, 11121–11132
17. Jónsson, Z. O., Hindges, R., and Hübscher, U. (1998) *The EMBO Journal* **17**, 2412–2425
18. Blanca, G., Shevelev, I., Ramadan, K., Villani, G., Spadari, S., Hübscher, U., and Maga, G. (2003) *Biochemistry* **42**, 7467–7476
19. García-Díaz, M., Bebenek, K., Sabariego, R., Domínguez, O., Rodríguez, J., Kirchhoff, T., García-Palomero, E., Picher, A. J., Juárez, R., Ruiz, J. F., Kunkel, T. A., and Blanco, L. (2002) *J Biol Chem* **277**, 13184–13191
20. Syvaaja, J., and Linn, S. (1989) *J Biol Chem* **264**, 2489–2497
21. Asturias, F. J., Cheung, I. K., Sabouri, N., Chilkova, O., Wepplo, D., and Johansson, E. (2006) *Nat. Struct. Mol. Biol.* **13**, 35–43
22. Brown, J. A., Pack, L. R., Sanman, L. E., and Suo, Z. (2011) *DNA Repair (Amst.)* **10**, 24–33
23. García-Díaz, M., Bebenek, K., Gao, G., Pedersen, L. C., London, R. E., and Kunkel, T. A. (2005) *DNA Repair (Amst.)* **4**, 1358–1367
24. Bork, P., Hofmann, K., Bucher, P., Neuwald, A. F., Altschul, S. F., and Koonin, E. V. (1997) *FASEB J.* **11**, 68–76
25. Fiala, K. A., Duym, W. W., Zhang, J., and Suo, Z. (2006) *J Biol Chem* **281**, 19038–19044
26. Efrati, E., Tocco, G., Eritja, R., Wilson, S. H., and Goodman, M. F. (1997) *J Biol Chem* **272**, 2559–2569
27. Bebenek, K., García-Díaz, M., Foley, M. C., Pedersen, L. C., Schlick, T., and Kunkel, T. A. (2008) *EMBO Rep* **9**, 459–464
28. Seki, M., Masutani, C., Yang, L. W., Schuffert, A., Iwai, S., Bahar, I., and Wood, R. D. (2004) *The EMBO Journal* **23**, 4484–4494
29. Daube, S. S., Tomer, G., and Livneh, Z. (2000) *Biochemistry* **39**, 348–355
30. García-Díaz, M., Bebenek, K., Larrea, A. A., Havener, J. M., Perera, L., Krahm, J. M., Pedersen, L. C., Ramsden, D. A., and Kunkel, T. A. (2009) *Nat. Struct. Mol. Biol.* **16**, 967–972
31. Lee, S. H., Pan, Z. Q., Kwong, A. D., Burgers, P. M., and Hurwitz, J. (1991) *J Biol Chem* **266**, 22707–22717
32. Chilkova, O., Stenlund, P., Isoz, I., Stith, C. M., Grabowski, P., Lundström, E., Burgers, P. M., and Johansson, E. (2007) *Nucleic Acids Res.* **35**, 6588–6597
33. Syvaaja, J., Suomensaaari, S., Nishida, C., Goldsmith, J. S., Chui, G. S., Jain, S., and Linn, S. (1990) *Proc Natl Acad Sci USA* **87**, 6664–6668
34. Kanuri, M., Minko, I. G., Nechev, L. V., Harris, T. M., Harris, C. M., and Lloyd, R. S. (2002) *J Biol Chem* **277**, 18257–18265
35. Maga, G., Villani, G., Ramadan, K., Shevelev, I., Tanguy Le Gac, N., Blanco, L., Blanca, G., Spadari, S., and Hübscher, U. (2002) *J Biol Chem* **277**, 48434–48440
36. Shivji, M. K., Podust, V. N., Hübscher, U., and Wood, R. D. (1995) *Biochemistry* **34**, 5011–5017
37. Araújo, S. J., Tirode, F., Coin, F., Pospiech, H., Syväoja, J. E., Stucki, M., Hübscher, U., Egly, J. M., and Wood, R. D. (2000) *Genes Dev.* **14**, 349–359
38. Ogi, T., Limsirichaikul, S., Overmeer, R. M., Volker, M., Takenaka, K., Cloney, R., Nakazawa, Y., Niimi, A., Miki, Y., Jaspers, N. G., Mullenders, L. H. F., Yamashita, S., Foustieri, M. I., and Lehmann, A. R. (2010) *Molecular Cell* **37**, 714–727
39. Dianov, G. L., Sleeth, K. M., Dianova, I. I., and Allinson, S. L. (2003) *Mutat. Res.* **531**, 157–163
40. Stucki, M., Pascucci, B., Parlanti, E., Fortini, P., Wilson, S. H., Hübscher, U., and Dogliotti, E. (1998) *Oncogene* **17**, 835–843
41. Sage, E., and Harrison, L. (2010) *Mutat. Res.*
42. Lopes, M., Foiani, M., and Sogo, J. M. (2006) *Molecular Cell* **21**, 15–27

43. Heller, R. C., and Mariani, K. J. (2006) *Nature* **439**, 557–562
44. Karras, G. I., and Jentsch, S. (2010) *Cell* **141**, 255–267

ACKNOWLEDGMENTS

This work was supported by a grant from EDF (Electricité de France) to GV and by grants 106986 and 123082 from the Academy of Finland to Hp and JES respectively. UH was supported by the University of Zurich and the Swiss National Science Foundation. We thank Dr Neil Johnson for critical reading of the manuscript.

FIGURES LEGENDS

Fig. 1. The ability of DNA polymerase λ to perform translesion synthesis of an AP site in the presence of DNA polymerase ϵ depends on the gap size.

Experiments were performed with templates shown in Table Ia. The enzymes and the DNA substrates used are indicated on top of the Figure: ss (single-stranded) stands for template-primer with no oligonucleotides hybridized downstream the AP site, while 2, 4, 6 and 13 indicate the length of gap regions between the AP site and the oligonucleotide hybridized downstream. Assays were carried out as described in Experimental procedures.

A) Lane 1: no polymerase present. Lanes 2, 5, 8, 11 and 14: reactions incubated for 35 minutes with 0.025 picomoles of pol ϵ . Lanes 3, 6, 9, 12 and 15: reactions incubated for 5 minutes with 0.25 picomoles of pol λ . Lanes 4, 7, 10, 13 and 16: reactions were incubated with 0.025 picomoles of pol ϵ for 30 minutes, then 0.25 picomoles of pol λ were added and incubation continued for 5 minutes.

The positions of the primer and of the AP site are indicated.

B) Quantification of the percentage of TLS calculated as described in Experimental procedures. Mean \pm S.D. values for three independent experiments are indicated. Light grey bars: pol ϵ alone. Dark grey bars: pols ϵ and λ together.

Fig. 2. DNA polymerase λ can extend both from a nucleotide preceding the AP site and from an A incorporated opposite to it when placed within a DNA gap, while DNA polymerase ϵ cannot.

Experiments were performed with the templates shown in Table Ic. The enzymes and the DNA substrates used are indicated on top of the Figures: ss stands for a template-primer with no oligonucleotides hybridized downstream the AP site, while 2, 4, 6 and 13 indicate the length of gap regions between the AP site and the oligonucleotide hybridized downstream. Assays were carried out as described in Experimental procedures.

A) Experiments conducted with the template primed with the 45 mer terminating one nucleotide before the AP site. Lane 1: no polymerase present. Lanes 2 to 6: reactions incubated for 35 minutes with 0.025 picomoles of pol ϵ . Lane 7: no polymerase present. Lanes 8 to 12: reactions incubated for 5 minutes with 0.25 picomoles of pol λ .

The positions of the primer and of the AP site are indicated.

B) Experiments conducted with the template primed with the 45 mer terminating with an A opposite to the AP site. Lane 1: no polymerase present. Lanes 2 to 6: reactions incubated for 35 minutes with 0.025 picomoles of pol ϵ . Lane 7: no polymerase present. Lanes 8 to 12: reactions incubated for 5 minutes with 0.25 picomoles of pol λ . The positions of the primer and of the AP site are indicated.

Fig. 3. Full length DNA polymerase λ is required for the AP site translesion synthesis in the presence of DNA polymerase ϵ .

Experiments were performed with templates shown in Table Ia. The enzymes and the DNA substrates used are indicated on top of the Figure: ss stands for template-primer with no oligonucleotides hybridized downstream the AP site, while 4 and 6 indicate the length of gap regions between the AP site and the oligonucleotide hybridized downstream. Assays were carried out as described in Experimental procedures.

A) Lane 1: no polymerase present. Lane 2: reaction incubated for 35 minutes with 0.025 picomoles of Pol ϵ . Lane 3: reaction incubated with 0.25 picomoles of pol λ for 5 minutes. Lane 4: reaction incubated with 0.25 picomoles of pol $\lambda^{244-575}$ for 5 minutes. Lane 5: reaction incubated with 0.025 picomoles of Pol ϵ for 30 minutes, then 0.25 picomoles of pol λ were added and incubation continued for 5 minutes. Lane 6: reaction incubated with 0.025 picomoles of pol ϵ for 30 minutes, then 0.25 picomoles of pol $\lambda^{244-575}$ were added and incubation continued for 5 minutes. Lanes 7 and 8: as for lanes 5 and 6 respectively.

The positions of the primer and of the AP site are indicated.

B) Quantification of the data from Fig. 3A, expressed as percentage of TLS calculated as described in Experimental procedures. White bar: pol ϵ alone. Dark grey bar: pol ϵ plus pol λ . Light grey bar: pol ϵ plus pol $\lambda^{244-575}$

Fig. 4. The capacity of the $\lambda^{244-575}$ truncated form of DNA polymerase λ to extend from the nucleotide preceding the AP site is impaired.

Experiments were performed with templates shown in Table Ic. The enzymes and the DNA substrates used are indicated on the top of the Figure: ss stands for template-primer with no oligonucleotides hybridized downstream the AP site, while 4, 6 and 13 indicate the length of gap regions between the AP site and the oligonucleotide hybridized downstream. Assays were carried out as described in Experimental procedures.

A) Lane 1: no polymerase present. Lane 2: reaction incubated for 5 minutes with 0.25 picomoles of pol λ . Lane 3: reaction incubated for 5 minutes with 0.25 picomoles of pol $\lambda^{244-575}$. Lane 4: reaction incubated for 5 minutes with 0.25 picomoles of pol λ . Lane 5: reaction incubated for 5 minutes with 0.25 picomoles of pol $\lambda^{244-575}$. Lane 6: reaction incubated for 5 minutes with 0.25 picomoles of pol λ . Lane 7: reaction incubated for 5 minutes with 0.25 picomoles of pol $\lambda^{244-575}$. Lane 8: reaction incubated for 5 minutes with 0.25 picomole of pol λ . Lane 9: reaction incubated for 5 minutes with 0.25 picomoles of pol $\lambda^{244-575}$.

The positions of the primer and of the AP site are indicated.

B) Quantification of the data from Fig. 4A, expressed as percentage of TLS calculated as described in Experimental procedures. Dark grey bar: pol λ . Light grey bar: pol $\lambda^{244-575}$

Fig. 5. DNA polymerase β favours smaller gap size than DNA polymerase λ in TLS of an AP site in the presence of DNA polymerase ϵ .

Experiments were performed with templates shown in Table Ia. The polymerases and the DNA substrates used are indicated on top of the Figure: ss stands for template-primer with no oligonucleotides hybridized downstream the AP site, while 2 and 4 indicate the length of gap regions between the AP site and the oligonucleotide hybridized downstream. Assays were carried out as described in Experimental procedures.

A) Lane 1: no polymerase present. Lane 2: reaction incubated for 35 minutes with 0.025 picomoles of pol ϵ . Lanes 3 and 4: reactions incubated with 0.25 picomoles of pol λ for 5 and 10 minutes respectively. Lanes 5 and 6: reactions incubated with 0.25 picomoles of pol β for 5 and 10 minutes respectively.

Lanes 7 and 8: reactions were incubated with 0.025 picomoles of pol ϵ for 30 minutes, then 0.25 picomole of pol λ were added and the incubation continued for 5 and 10 minutes respectively. Lanes 9, 10 and 11, 12: as for lanes 7 and 8. Lanes 13 and 14: reactions were incubated with 0.025 picomoles of pol ϵ for 30 minutes, then 0.25 picomoles of pol β were added and the incubation continued for 5 and 10 minutes respectively. Lanes 15, 16 and 17, 18: as for lanes 13 and 14. The positions of the primer and of the AP site are indicated.

B) Quantification of the data from Fig. 5A with pol λ , expressed as percentage of TLS calculated as described in Experimental procedures.

White bar: pol ϵ alone. Light grey bars: incubation with pol ϵ and then pol λ added for 5 minutes. Dark grey bars: incubation with pol ϵ and then pol λ added for 10 minutes.

C) Quantification of the data from Fig. 5A with pol β , expressed as percentage of TLS and calculated as described in Experimental procedures.

Light grey bars: incubation with pol ϵ and pol β added for 5 minutes. Dark grey bars: incubation with pol ϵ and pol β added for 10 minutes.

Fig. 6. Influence of DNA gap sizes on translesion synthesis of an AP site by DNA polymerases λ and η . Experiments were performed with templates shown in Table I. The enzymes and the DNA substrates used are indicated on top of the Figure: ss (single-stranded) stands for template-primer with no oligonucleotides hybridized downstream the AP site, while 1, 2, 4, and 6 indicate the length of gap regions between the AP site and the oligonucleotide hybridized downstream. Assays were carried out as described in Experimental procedures.

A) Experiments with templates in Table Ia. Lane 1: no polymerase present. Lane 2: reaction incubated for 35 minutes with 0.025 picomoles of pol ϵ . Lane 3: reaction incubated for 5 minutes with 0.25 picomoles of pol λ . Lane 4: reaction incubated for 5 minutes with 0.25 picomoles of pol η . Lanes 5, 6, 7 and 8: reaction was incubated with 0.025 picomoles of pol ϵ and then 0.25 picomoles of pol λ were added and the reaction continued for 5 minutes. Lanes 9, 10, 11 and 12: reaction was incubated with 0.025 picomoles of pol ϵ and then 0.25 picomoles of pol η were added and the reaction continued for 5 minutes.

B) Experiments with templates in Table 1C primed with the 45 mer terminating one nucleotide before the AP site. Lanes 1 and 12: no polymerase present. Lanes 2, 3, 4, 5 and 6 reactions incubated 5 minutes with pol λ . Lanes 7, 8, 9, 10 and 11 reactions incubated 5 minutes with pol η .

The positions of the primers, of the AP site and of the 100 mer full length product are indicated.

Fig. 7. PCNA and RPA have no effect on gap size specificity of TLS of an AP site by DNA polymerase λ in presence of DNA polymerase ϵ .

Experiments were performed with templates shown in Table Ia. The proteins and the DNA substrates used are indicated on the top of the Figure: ss stands for template-primer with no oligonucleotides hybridized downstream the AP site, while 1, 2 and 4 6, 8, 10 and 13 indicate the length of gap regions between the AP site and the oligonucleotide hybridized downstream. Assays were carried out as described in Experimental procedures.

A) Lane 1: no polymerase present. Lane 2: reaction incubated for 35 minutes with 0.025 picomoles of pol ϵ , 1.2 picomoles of PCNA and 0.25 picomoles of RPA. Lanes 3 to 10: the reactions were incubated for 30 minutes with 0.025picomole of pol ϵ , 1.2 picomoles of PCNA and 0.25 picomoles of RPA, then 0.25 picomoles of pol λ were added and the incubation continued for 5 minutes. The positions of the primer and of the AP site are indicated.

B) Quantification of data from Fig. 6A, expressed as percentage of TLS calculated as described in Experimental procedures. White bar: reaction without pol λ . Grey bar: complete reaction

Fig. 8. PCNA and RPA have no effect on gap size specificity of TLS of an AP site by DNA polymerase β in the presence of DNA polymerase ϵ .

Experiments were performed with templates shown in Table Ia. The proteins and the DNA substrates used are indicated on the top of the Figure: ss stands for template-primer with no oligonucleotides hybridized downstream the AP site, while 1, 2 and 4 6, 8, 10 and 13 indicate the length of gap regions between the AP site and the oligonucleotide hybridized downstream. Assays were carried out as described in Experimental procedures.

A) Lane 1: reaction incubated for 35 minutes with 0.025 picomoles of pol ϵ , 1.2 picomoles of PCNA and 0.25 picomoles of RPA. Lanes 2 to 9: the reactions were incubated for 30 minutes with 0.025 picomoles of pol ϵ , 1.2 picomoles of PCNA and 0.25 picomoles of RPA, then 0.25 picomoles of pol β were added and the incubation continued for 5 minutes. The positions of the primer and of the AP site are indicated.

B) Quantification of data from Fig. 7A, expressed as percentage of TLS calculated as described in Experimental procedures. White bar: reaction without pol β . Grey bar: complete reaction.

Fig. 9. Tentative model of TLS over an AP site by DNA polymerases λ and β in the presence of DNA polymerase ϵ .

For details see text.

Table I – DNA templates

a)

3' ATTCCATCATCATAATATTT ◀ 1-13 gap ▶
 3' ATTCCATCATCATAATATTTAAT
 3' ATTCCATCATCATAATATTTAATAC
 3' ATTCCATCATCATAATATTTAATACC
 3' ATTCCATCATCATAATATTTAATACCT
 3' ATTCCATCATCATAATATTTAATACCTC
 3' ATTCCATCATCATAATATTTAATACCTCC
 3' ATTCCATCATCATAATATTTAATACCTCCA
 3' ATTCCATCATCATAATATTTAATACCTCCAA
 3' ATTCCATCATCATAATATTTAATACCTCCAAA TCTAATAATCCTAACTTTAATACATTAACTTTCATTTACATCA 5'
 5' TAAGGTAGTAGTATTATAAATTATGGAGGTTTTXAGTGGGAAATAAAATATAGTAAAGATTATTAGGATTTGAAATTATGTAATTGAAAGTAAATGTAGT 3'

b)

3' ATTCCATCATCATAATATTT
 3' ATTCCATCATCATAATATTTAATACCTCC TCTAATAATCCTAACTTTAATACATTAACTTTCATTTACATCA 5'
 5' TAAGGTAGTAGTATTATAAATTATGGAGGTTTTXACTCCCAAATAAAATATACTCCAGATTATTAGGATTTGAAATTATGTAATTGAAAGTAAATGTAGT 3'

c)

3' ATTCCATCATCATAATATTT
 3' ATTCCATCATCATAATATTTAATACCT
 3' ATTCCATCATCATAATATTTAATACCTCC TCACCCTTTATTTTATATCATTTCTAATAATCCTAACTTTAATA 5'
 3' ATTCCATCATCATAATATTTAATACCTCCAA ATCACCCTTTATTTTATATCATTTCTAATAATCCTAACTTTAAT 5'
 5' TAAGGTAGTAGTATTATAAATTATGGAGGTTTTXAGTGGGAAATAAAATATAGTAAAGATTATTAGGATTTGAAATTATGTAATTGAAAGTAAATGTAGT 3'

d)

TCACCCTTTATTTTATATCATTTCTAATAATCCTAACTTTAATACATTAACTTTCATTTACATCA 5'
 5' TAAGGTAGTAGTATTATAAATTATGGAGGTTTTXAGTGGGAAATAAAATATAGTAAAGATTATTAGGATTTGAAATTATGTAATTGAAAGTAAATGTAGT 3'

X indicates the synthetic abasic site on the damaged template or a guanine on the undamaged template.

All the oligonucleotides complementary to the 5' end of the 100-mer templates bear a 5' phosphate.

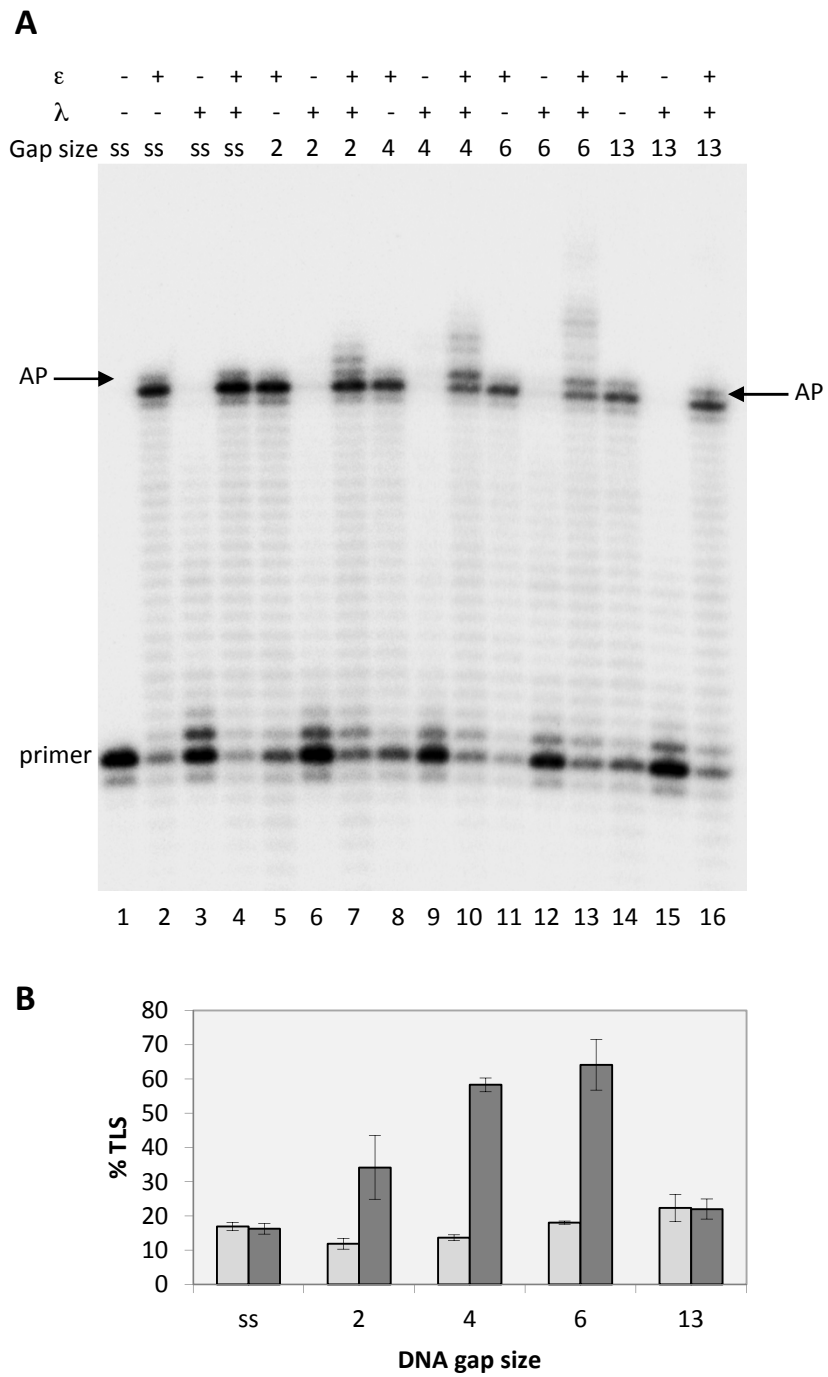


Fig.1

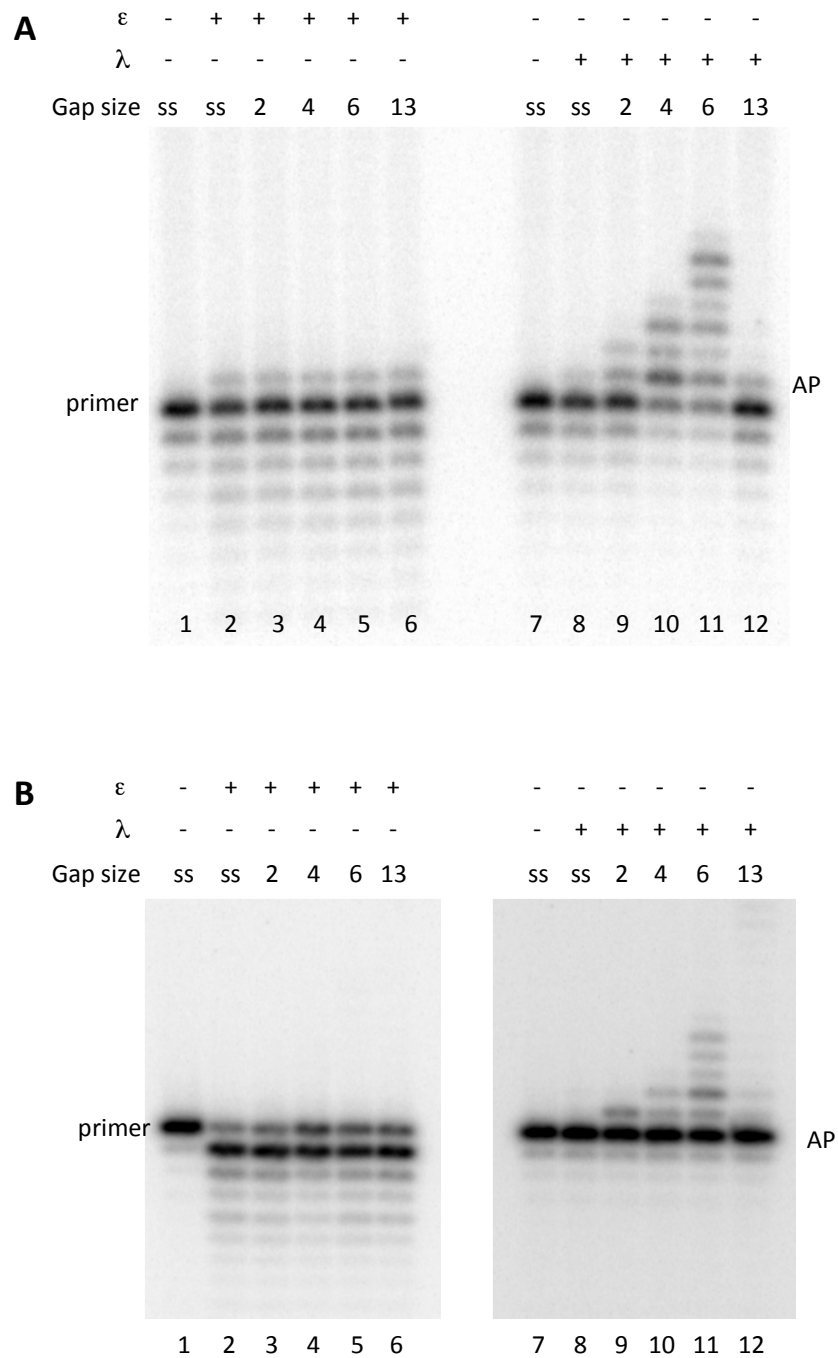


Fig.2

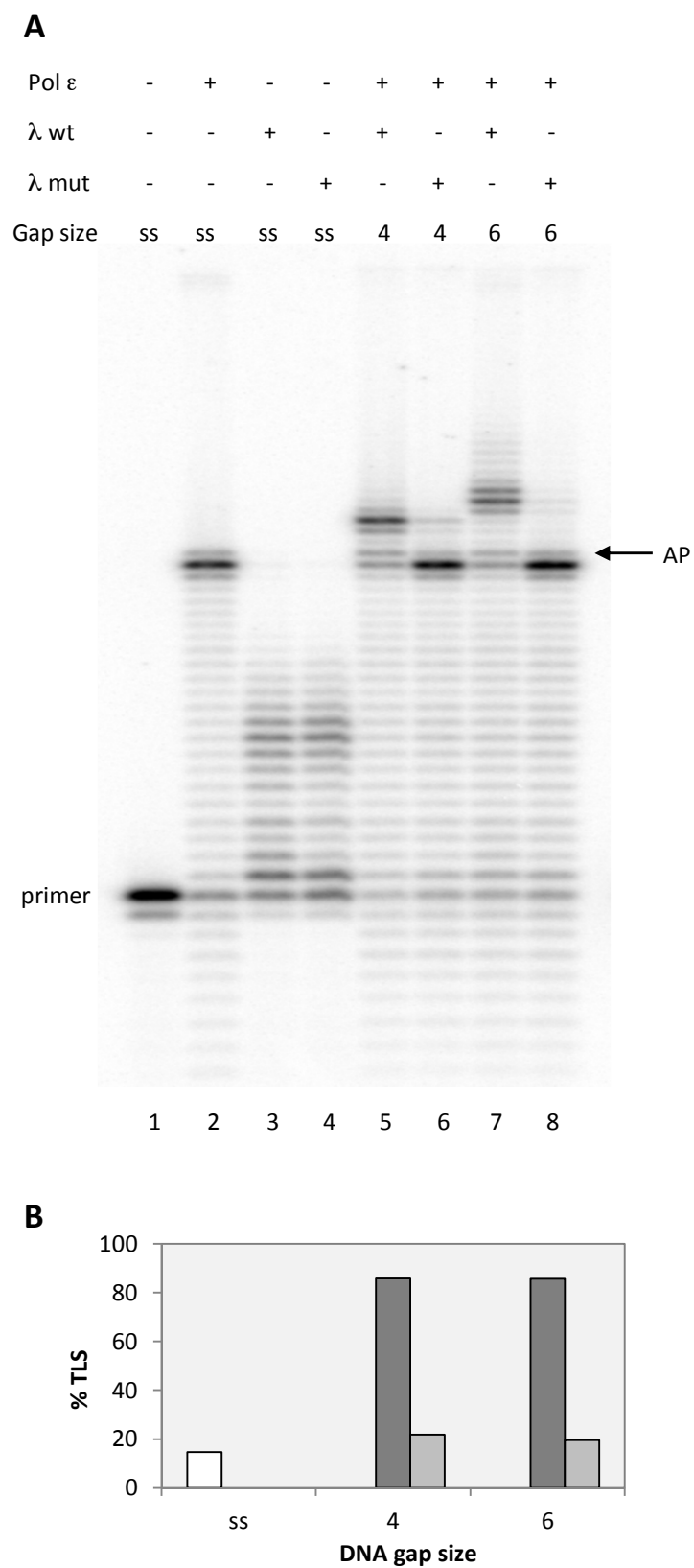


Fig. 3

A

| | | | | | | | | | |
|---------------|----|----|----|---|---|---|---|----|----|
| λ wt | - | + | - | + | - | + | - | + | - |
| λ mut | - | - | + | - | + | - | + | - | + |
| Gap size | ss | ss | ss | 4 | 4 | 6 | 6 | 13 | 13 |

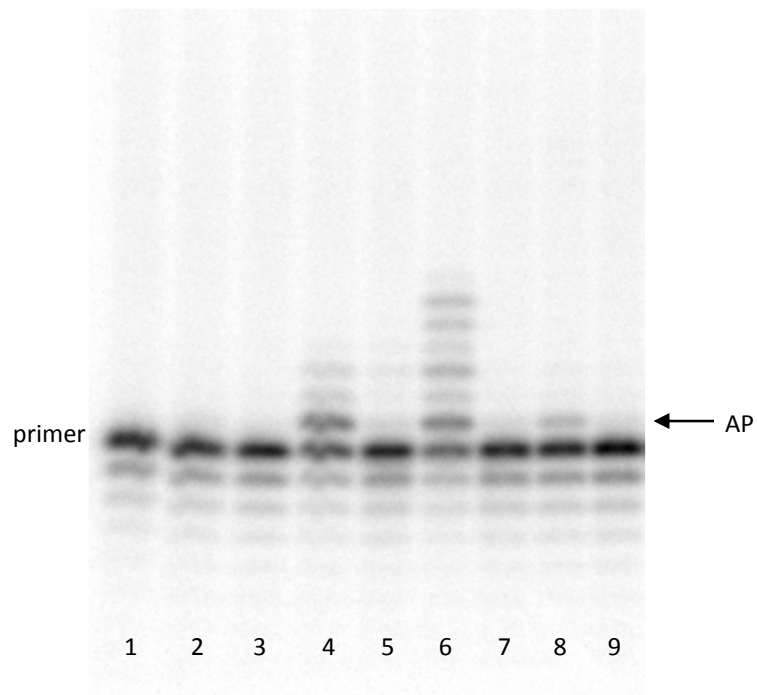
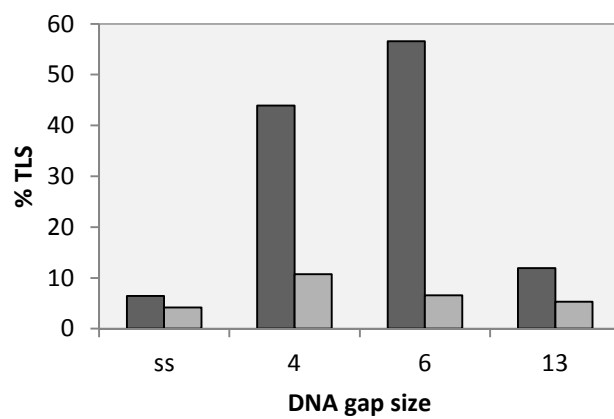
**B**

Fig. 4

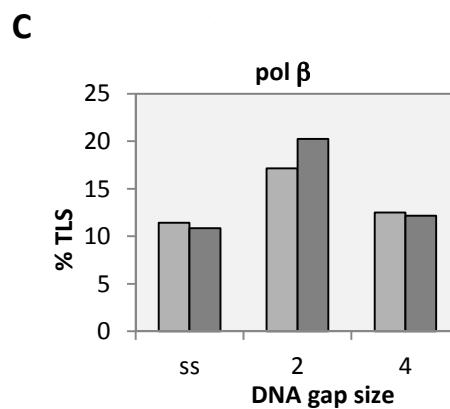
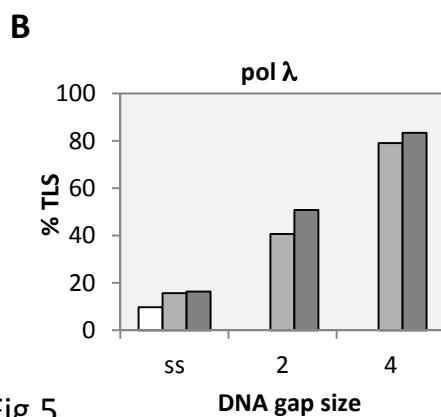
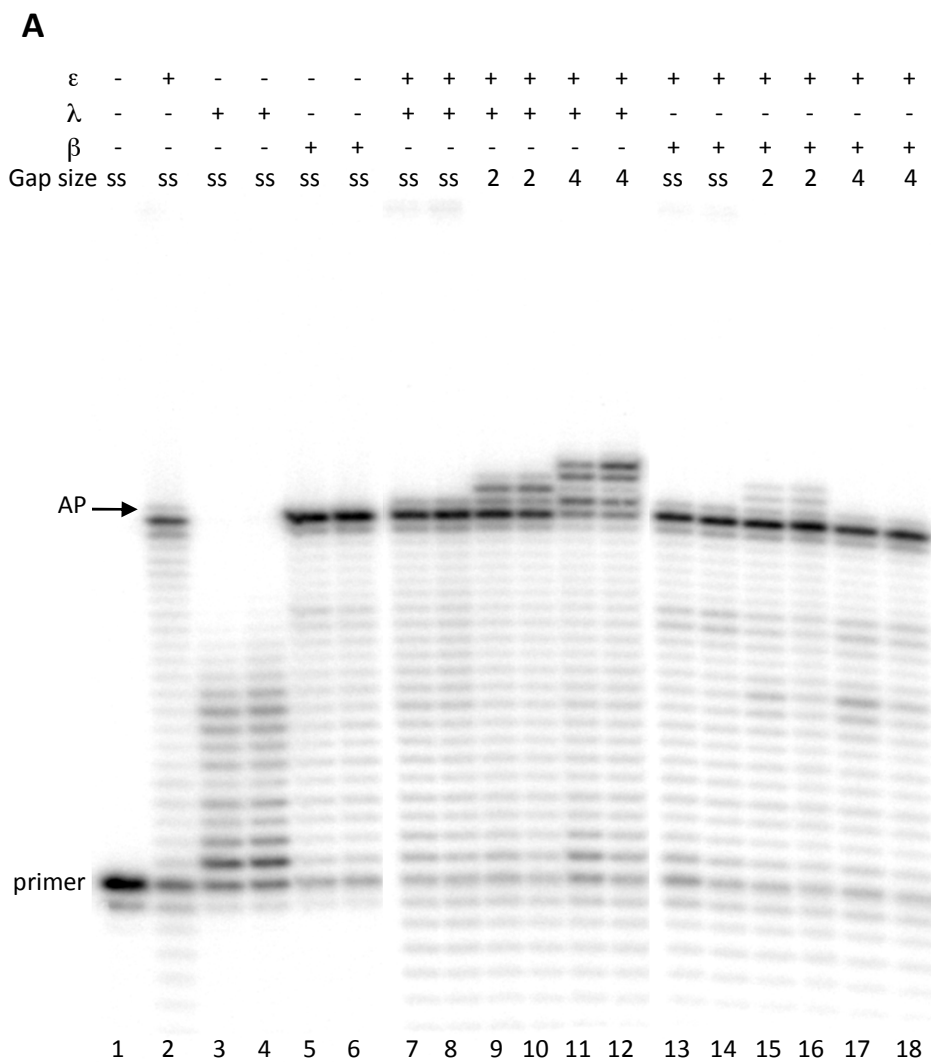
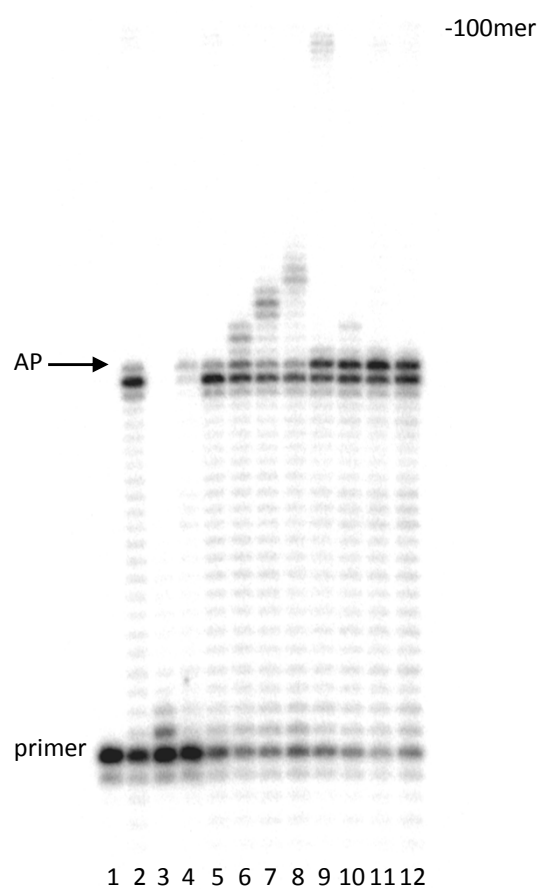


Fig.5

A

| | | | | | | | | | | | | |
|------------|----|----|----|----|----|---|---|---|----|---|---|---|
| ϵ | - | + | - | - | + | + | + | + | + | + | + | + |
| λ | - | - | + | - | + | + | + | + | - | - | - | - |
| η | - | - | - | + | - | - | - | - | + | + | + | + |
| Gap size | ss | ss | ss | ss | ss | 2 | 4 | 6 | ss | 2 | 4 | 6 |



B

| | | | | | | | | | | | | |
|-----------|----|----|---|---|---|---|----|---|---|---|---|----|
| λ | - | + | + | + | + | + | - | - | - | - | - | - |
| η | - | - | - | - | - | - | + | + | + | + | + | - |
| Gap size | ss | ss | 1 | 2 | 4 | 6 | ss | 1 | 2 | 4 | 6 | ss |

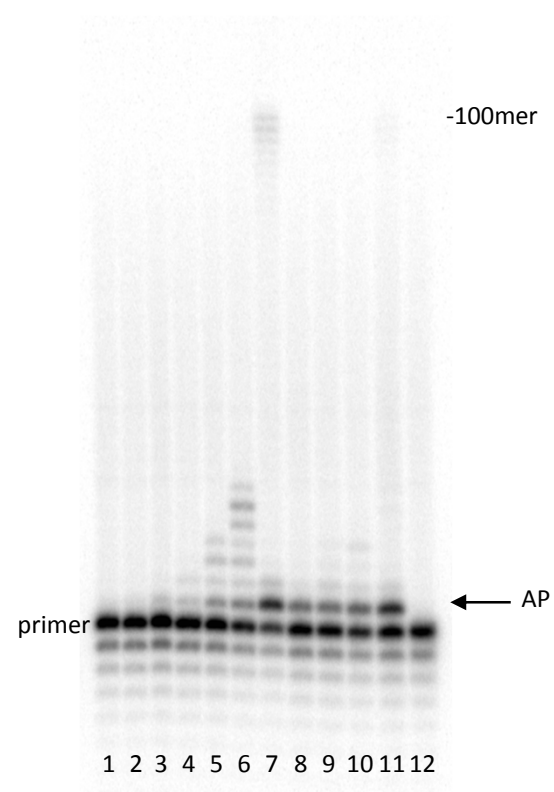


Fig.6

A

| | | | | | | | | | | |
|------------|----|----|----|---|---|---|---|---|----|----|
| ϵ | - | + | + | + | + | + | + | + | + | + |
| PCNA | - | + | + | + | + | + | + | + | + | + |
| RPA | - | + | + | + | + | + | + | + | + | + |
| λ | - | - | + | + | + | + | + | + | + | + |
| Gap size | ss | ss | ss | 1 | 2 | 4 | 6 | 8 | 10 | 13 |

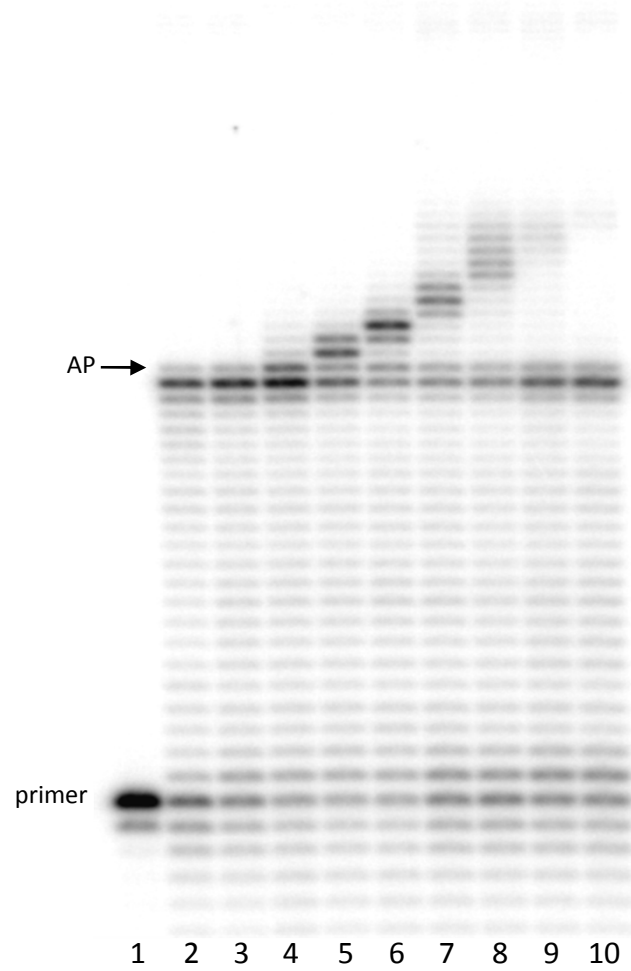
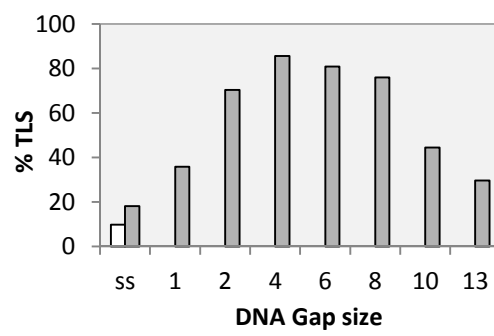
**B**

Fig.7

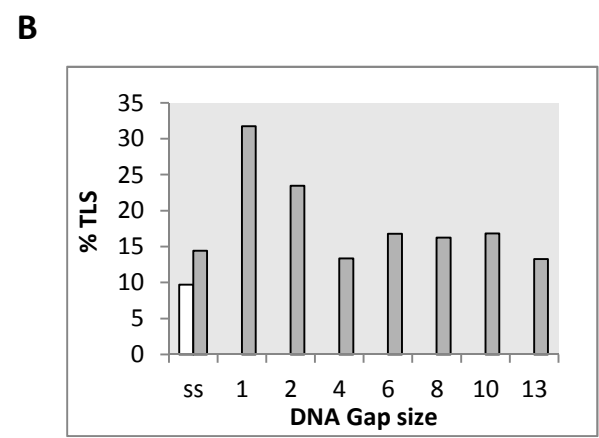
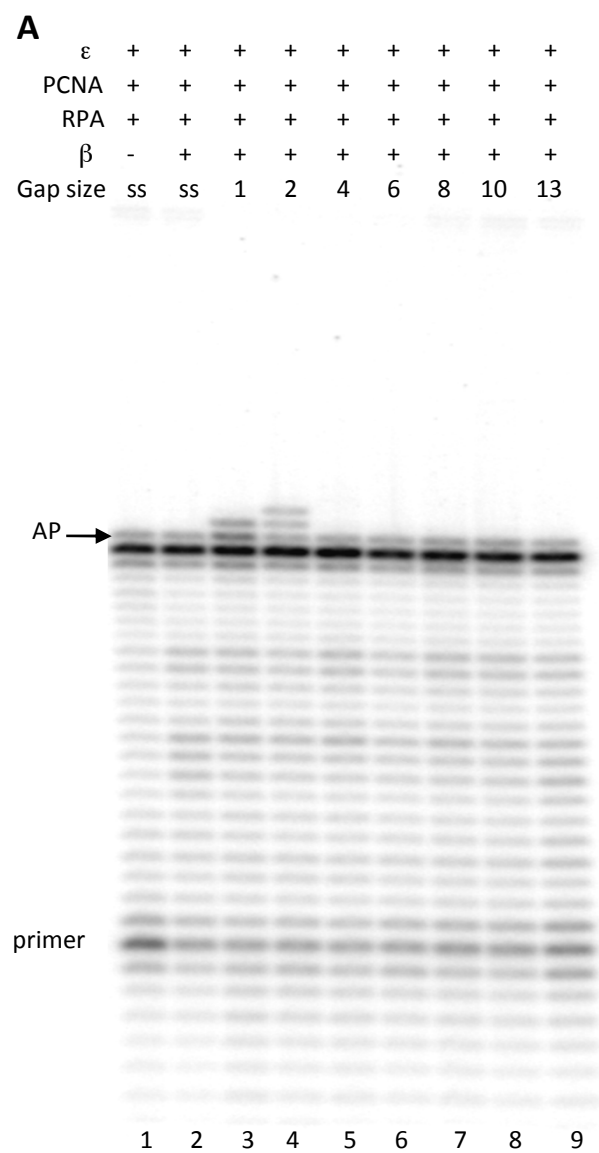


Fig.8

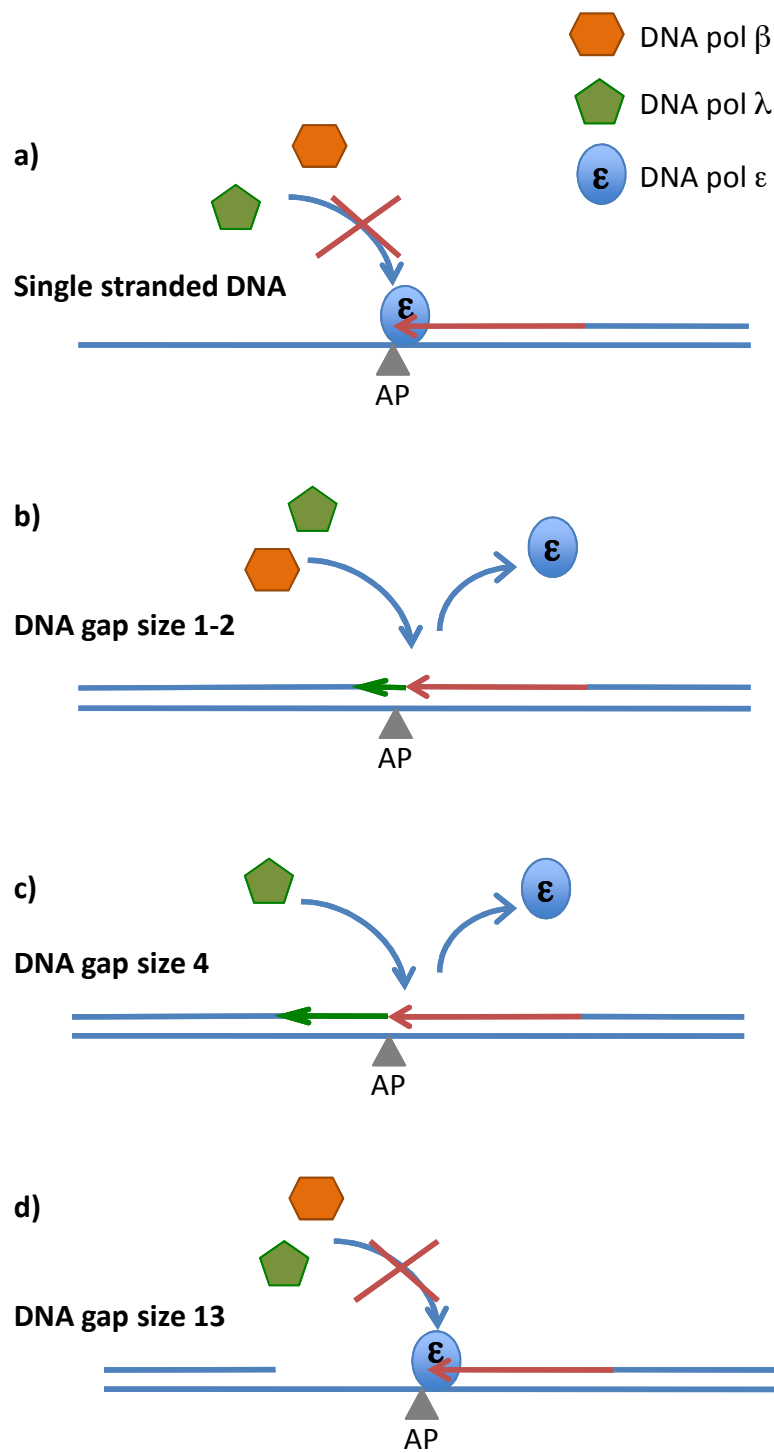


Fig.9